Nucleotides and nucleosides in ovine and caprine milk during lactation

S. Plakantara,* A.-M. Michaelidou,* A. Polychroniadou,*¹ G. Menexes,† and E. Alichanidis*

*Department of Food Science and Technology, and

†Department of Field Crops and Ecology, School of Agriculture, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece

ABSTRACT

The aim of this study was to determine the nucleoside and nucleotide content in ovine and caprine milks at the colostral, transitional, and mature stages of lactation. Samples from 18 dairy sheep and 18 dairy goats were collected at 1, 2, 3, 4, 5, and 15 d postpartum. Separation and quantitation of the 5'-nucleotides (NT) and the nucleosides (NS) was performed by reverse phase HPLC. For each compound measured, considerable interindividual variation was recorded in both species of milk. The total NS content ranged from 57 to 132 $\mu\text{mol/L}$ and from 54 to 119 $\mu\text{mol/L}$ in ovine and caprine milk, respectively. The major NS identified in both species of milk was uridine, representing more than 60% of the total NS pool. The mean levels of inosine and guanosine were comparable between ewe and goat milk. Instead, the mean level of cytidine across the sampling period was much higher in ewe milk (11.9 $\mu\text{mol/L}$ compared with 4.5 $\mu\text{mol/L}$ in goat milk) and exhibited a peak value on the fourth day of lactation. The adenosine content was at least 3-fold higher in caprine milk compared with its ovine counterpart. The total NS and orotic acid contents did not differ significantly between the 2 species. However, in the case of total NT content, interspecies differences were significant, with NT levels ranging from 294 to 441 $\mu\text{mol/L}$ in ovine milk and from 166 to 366 $\mu\text{mol/L}$ in caprine milk. The NT content in colostrum (1–3 d) of both species was higher than in mature milk (15 d), and uridine monophosphate was the dominant NT in all samples.

Key words: nucleotide, nucleoside, sheep milk, goat milk

INTRODUCTION

As nutrition science moves beyond the study of essential nutrients, considerable research interest is focused on minor components of the non-protein–nitrogen fraction of milk that may be able to transmit biochemical

messages with significant health implications (Michaelidou and Steijns, 2006; Michaelidou, 2008). As such, nucleotides (NT) have attracted particular scientific attention because they are ubiquitous intracellular compounds of crucial importance to cellular function and metabolism. The work conducted by Cosgrove (1998) on the biological role of these compounds indicated their relation with the following 5 main areas: immune function, iron absorption, lipid metabolism, intestinal flora, and intestinal and hepatic morphology and function. Nucleotides can be synthesized endogenously and are, therefore, not considered essential nutrients (Sánchez-Pozo and Gil, 2002). However, investigations in human and animal models suggest that dietary NT may become essential when the endogenous supply is insufficient for normal function, even though their absence from the diet does not lead to a classic clinical deficiency syndrome. Conditions under which these nutrients may become essential include certain disease states, periods of limited nutrient intake or rapid growth, and the presence of regulatory or developmental factors that interfere with full expression of the endogenous synthetic capacity. Under these conditions, the dietary intake of the nutrient spares the organism the cost of de novo synthesis or salvage and may optimize tissue function (Carver, 1999).

During the last 3 decades, numerous studies have dealt with the occurrence of NT and their metabolites in human milk, given the functional role that these components play in neonatal nutrition (Gil and Sanchez-Medina, 1982; Janas and Picciano, 1982; Leach et al., 1995; Thorell et al., 1996; Duchén and Thorell, 1999). However, fewer studies have been devoted to bovine NT profile (Tiemeyer et al., 1984; Schlimme et al., 1997, 2000; Ferreira, 2003) and an even more limited number to the content and distribution of NT and nucleosides (NS) in ovine and caprine milk (Schlimme et al., 1997; Martin et al., 2005).

Although about 84% of milk worldwide is produced by cows (IDF, 2008), the contribution of milk from other domesticated animals to the survival and well-being of people around the world is immense and invaluable, especially in areas where cow breeding is difficult because of adverse environmental conditions.

Received October 20, 2009.

Accepted February 9, 2010.

¹Corresponding author: annapoly@agro.auth.gr

Greece is one of the leading ovine and caprine milk producers in the Mediterranean region and the leading producer in Europe. Caprine and ovine milk compose more than 60% of Greek milk production, reaching the amount of 1.25 million tonnes annually (IDF, 2008). As a consequence, these milk species are considered a fundamental and indispensable part of the Greek and Mediterranean diet.

The potential value of using ovine or caprine milk or the respective colostral fractions in clinical nutrition is gathering momentum because of the important role their nonpeptide trophic factors, such as nucleotides and nucleosides, could play in homeostatic regulation. In particular, these factors can help to maintain gastrointestinal mucosal mass and modulate the immune system via multiple mechanisms (e.g., altering intestinal flora and influencing the actions of growth factors; Playford et al., 2000). It is worth noting that because of the bio- and trophochemical properties of dietary nucleotides and nucleosides, the European Commission has permitted the use of supplementation with specific ribonucleotide salts in the manufacture of infant and follow-on formulas (EC, 1996). Therefore, besides the attractive value to different clinical situations, the opportunity to use ovine and caprine colostrum and milk to develop problem-oriented supplementation packages for preterm neonates and infants that are small for gestational age remains an appealing area of research.

The present study was motivated by the nutritional significance of these compounds for specific population groups and the economic impact of ovine and caprine milk production in Greece and other Mediterranean countries. Thus, the objective was to identify and quantify free NT and NS in ovine and caprine milk from indigenous Greek breeds sampled at different stages of lactation.

MATERIALS AND METHODS

Sample Preparation

Colostrum and milk were collected from 18 ewes of the Serron breed and 18 local-breed goats. Sampling was performed at 1, 2, 3, 4, 5, and 15 d after parturition. Samples were flushed with N₂ and frozen immediately after collection and were then stored at -25°C until analyzed.

Sample preparation was performed as follows. Equal volumes of milk and 13% (wt/vol) perchloric acid (Merck, Darmstadt, Germany) were mixed for 10 min at room temperature using a magnetic stirrer. Before mixing, colostrum samples from 1 and 2 d postpartum were diluted 1:1 with distilled water. The precipitate was separated by centrifugation at 7,000 × *g* for 15 min

at 4°C. The pH of 25 mL of the supernatant was slowly adjusted to 4.00 using 5 *M* KOH (Panreac, Barcelona, Spain) and the volume was brought to 50 mL using double-distilled water. Samples were kept for 1 h in an ice bath. A portion of 2.5 mL was then filtered through 0.2-μm cellulose acetate filters (Alltech Assoc. Inc., Deerfield, IL) and used for analysis. All reagents employed for the extraction step were of analytical grade.

Standard Solutions

Analytical grade nucleotides [adenosyl-5'-monophosphate (**AMP**), cytidyl-5'-monophosphate (**CMP**), uridyl-5'-monophosphate (**UMP**), and guanosyl-5'-monophosphate (**GMP**)], nucleosides (cytidine, uridine, inosine, guanosine, and adenosine), and orotic acid (Sigma, St. Louis, MO) were used for preparation of standard solutions. The solutions were filtered through 0.2-μm cellulose acetate filters (Alltech Assoc. Inc.) and stored at 4°C.

Chromatographic Analysis

Separation and quantitation of the 5'-nucleotides and the NS was performed by reverse phase-HPLC using a binary solvent system (LKB, Bromma, Sweden) in conjunction with a Nucleosil C₁₈ column (120–5 μm, 250 × 4 mm; Macherey-Nagel, Düren, Germany) and a guard column (40 × 4 mm). Solvent A was 0.15 *M* KH₂PO₄, pH 4.00, and solvent B was 25% (vol/vol) acetonitrile in solvent A. The elution was conducted at room temperature at a flow-rate of 0.8 mL/min, with a linear gradient from 0 to 20% (vol/vol) solvent B for 20 min followed by a linear gradient from 20 to 100% (vol/vol) solvent B for 5 min, a linear gradient from 100 to 0% (vol/vol) solvent B for 1 min, and an isocratic elution with solvent A for 9 min. The absorbance of the eluate was monitored at both 254 and 278 nm using a programmable UV/visible detector (Fasma 525, Linear Instruments, Reno, NV), which was linked to a data acquisition and processing system (Nelson Analytical Inc., Paramus, NJ). The determination of orotic acid was performed using the same system under isocratic conditions for 5 min, with the mobile phase being 0.1 *M* trisodium citrate dihydrate (C₆H₅Na₃O₇·2H₂O), pH 6.50, containing 8 mL/L of acetonitrile; the flow-rate was 0.8 mL/min, and the absorbance was monitored at 278 nm.

All solvents were filtered through 0.45-μm Nylon 66 filters (Alltech Assoc. Inc.) before chromatographic analysis. Each compound was identified by its retention time when coinjected with the standards. Quantitation was carried out by use of external standard calibration; 5 concentrations were used for plotting the calibration

Table 1. Analysis of variance *F*-values and significance of model effects for the 12 measured traits in ovine and caprine milk samples during lactation¹

Item	Milk species		Lactation day		MS × LD	
	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
Nucleosides ²						
Cytidine	170.862	<0.001	15.814	<0.001	16.705	<0.001
Uridine	0.755	0.391	34.738	<0.001	49.429	<0.001
Inosine	1.931	0.174	12.538	<0.001	6.376	<0.001
Guanosine	4.057	0.052	15.037	<0.001	7.973	<0.001
Adenosine	230.480	<0.001	7.528	<0.001	22.079	<0.001
Total NS	0.076	0.784	24.651	<0.001	50.474	<0.001
Nucleotides ³						
CMP	62.135	<0.001	19.045	<0.001	8.422	<0.001
UMP	31.294	<0.001	33.900	<0.001	5.148	<0.001
GMP	0.082	0.776	17.034	<0.001	7.267	<0.001
AMP	5.614	0.024	15.358	<0.001	13.462	<0.001
Total NT	37.610	<0.001	37.907	<0.001	5.357	<0.001
Orotic acid	0.065	0.800	43.995	<0.001	12.942	<0.001

¹Milk species: *F* (1, 34); lactation day: *F* (5, 170); milk species × lactation day (MS × LD): *F* (5, 170).

²Total NS: total content of all nucleosides measured.

³CMP: cytidyl-5'-monophosphate; UMP: uridyl-5'-monophosphate; GMP: guanosyl-5'-monophosphate; AMP: adenosyl-5'-monophosphate; total NT: total content of all nucleotides measured.

curves for each compound. For quantitation, the coelution of orotic acid and CMP was alleviated by monitoring both compounds at 254 nm and also monitoring orotic acid at 278 nm. Single-compound standard solutions, containing either compound, were monitored at both wavelengths and the respective response factors were calculated; CMP does not absorb at 278 nm, thus allowing the direct quantitation of orotic acid at this wavelength.

Statistical Analysis

The experimental data were analyzed by ANOVA according to the linear model, which involves 1 factor between (animal species: sheep and goats) and 1 factor within (repeated measures at 6 sampling times postpartum) experimental units; this analysis is equivalent to the analysis of a split-plot design (Gomez and Gomez, 1984). Prior to the ANOVA, the normality and the homogeneity of variance assumptions were tested. Because of violations of the aforementioned assumptions, all measures (*X*) were $\log_{10}(X + 1)$ transformed to achieve normality and homogeneity between the levels of the 2 factors examined (Gomez and Gomez, 1984). Differences between means were compared by using the least significant difference test. All statistical analyses were performed by SPSS statistical software (version 15.0, SPSS Inc., Chicago, IL). The significance level of all hypothesis testing procedures was preset at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The ANOVA of cytidine, uridine, inosine, guanosine, adenosine, and total NS data, along with the corre-

sponding nucleoside-5'-monophosphates (CMP, UMP, GMP, AMP, and total NT) and orotic acid data, indicated significant differences in some of the parameters studied. These differences are attributed partly to the milk species (sheep, goat), but mostly to the lactation day and their interaction (Table 1). The effect of lactation day on NS, NT, and orotic acid content is shown in Tables 2 and 3. A typical HPLC chromatogram from the analysis of a mixed standard solution of NT and NS is presented in Figure 1.

Nucleosides

Milk species had a significant effect only on the cytidine and the adenosine determined ($P < 0.001$), whereas it did not have any effect on the other parameters studied ($P_{\text{uridine}} = 0.391$, $P_{\text{inosine}} = 0.174$, $P_{\text{guanosine}} = 0.052$, and $P_{\text{total NS}} = 0.784$; Table 1). Mean concentrations across lactation days are given in Table 2.

Both the lactation day and the milk species × lactation day interaction had a significant effect ($P = 0.001$) on all NS parameters measured (Table 1). In particular, the interaction of uridine, cytidine, and adenosine concentrations clearly indicate that the sheep and goat milk composition was affected differently by the lactation day, although at 1 d after parturition the levels of all 3 NS were almost comparable between the 2 milk species (Table 2; Figure 2). Among all NS identified, the uridine concentration in sheep milk increased with increasing lactation day, whereas its concentration in goat milk increased until 2 d after parturition and decreased after that. The similar trends observed for uridine and total NS (Figure 2) simply reflect the fact

Table 2. Effect of lactation day (LD) on nucleoside (NS) concentration [$\log_{10}(X + 1)$ transformed values] in sheep and goat milk ($\mu\text{mol/L}$)¹

Milk species	LD ²	Cytidine	Uridine	Inosine	Guanosine	Adenosine	Total NS ³
Sheep (n = 18)	1	0.993 (9.11)	1.524 (34.41)	0.910 (7.82)	0.284 (0.99)	0.773 (5.01)	1.756 (57.33)
	2	0.949 (8.92)	1.621 (42.17)	0.726 (5.03)	0.204 (0.80)	0.589 (3.20)	1.773 (60.12)
	3	1.039 (10.82)	1.863 (74.97)	0.858 (6.61)	0.511 (2.35)	0.576 (2.95)	1.980 (97.70)
	4	1.300 (20.55)	1.775 (64.03)	0.557 (2.91)	0.446 (2.09)	0.538 (2.59)	1.948 (92.17)
	5	1.106 (14.59)	1.961 (94.71)	0.709 (4.71)	0.461 (2.01)	0.315 (1.21)	2.054 (117.23)
	15	0.852 (7.38)	2.051 (115.26)	0.796 (6.56)	0.323 (1.16)	0.423 (1.91)	2.112 (132.27)
	Mean	1.040 (11.90)	1.799 (70.93)	0.759 (5.60)	0.372 (1.57)	0.536 (2.81)	1.937 (92.80)
Goat (n = 18)	1	0.938 (8.55)	1.660 (46.39)	1.014 (9.62)	0.220 (0.69)	0.810 (5.88)	1.848 (71.13)
	2	0.778 (5.19)	1.976 (96.48)	0.848 (6.59)	0.416 (1.63)	0.969 (9.19)	2.072 (119.08)
	3	0.746 (4.63)	1.963 (92.69)	0.738 (4.94)	0.394 (1.62)	1.057 (10.98)	2.057 (114.86)
	4	0.641 (3.48)	1.893 (81.72)	0.779 (5.43)	0.325 (1.35)	0.995 (9.59)	1.992 (101.68)
	5	0.608 (3.11)	1.860 (72.99)	0.798 (5.63)	0.421 (1.77)	0.988 (8.90)	1.963 (92.40)
	15	0.507 (2.26)	1.585 (39.01)	0.670 (3.87)	0.201 (0.63)	0.968 (8.56)	1.732 (54.33)
	Mean	0.703 (4.54)	1.823 (71.55)	0.808 (6.03)	0.330 (1.28)	0.965 (8.85)	1.944 (92.25)
CV (%) ⁴		(61.7)	(24.3)	(44.1)	(63.0)	(34.0)	(20.5)
LSD _{0.05} ⁴		0.110	0.090	0.123	0.092	0.101	0.075

¹The values in parentheses correspond to the respective raw data means.²Mean: mean concentrations across lactation days.³Total NS: total content of all nucleosides measured.⁴The comparison of means was performed using the $\log_{10}(X + 1)$ transformed values.

that the mean uridine concentration across lactation days is approximately 75% of the total NS content. The cytidine levels in sheep milk tended to increase during the colostrum phase, exhibited a distinct maximum concentration at 4 d postpartum, and decreased significantly after that. In contrast, the cytidine content progressively decreased with advancing lactation in the case of goat milk. The latter trend was also followed by adenosine in sheep milk, whereas its concentration in

goat milk was increased for the first 3 lactation days and decreased slightly thereafter, demonstrating a fairly constant level subsequent to the colostrum phase. Guanosine concentration increased in essence for the first 3 lactation days, regardless of milk species, and decreased after 5 d. Inosine in goat milk decreased up to the third day of lactation and demonstrated a fairly constant level until the fifth day and a decreasing trend toward the fifteenth day. Instead, its concentration in

Table 3. Effect of lactation day (LD) on nucleotide (NT) concentration [$\log_{10}(X + 1)$ transformed values] in sheep and goat milk ($\mu\text{mol/L}$)^{1,2}

Milk species	LD ³	CMP	UMP	GMP	AMP	Total NT	Orotic acid
Sheep (n = 18)	1	1.474 (30.52)	2.547 (362.83)	0.861 (6.72)	1.227 (17.55)	2.610 (417.61)	1.921 (86.50)
	2	1.358 (22.55)	2.591 (393.46)	0.852 (6.35)	1.252 (18.30)	2.642 (440.66)	1.992 (103.06)
	3	1.493 (31.80)	2.573 (379.01)	1.181 (14.81)	1.179 (15.69)	2.641 (441.30)	2.098 (132.06)
	4	1.507 (32.45)	2.433 (286.22)	1.030 (11.14)	1.061 (13.34)	2.514 (343.15)	1.816 (71.96)
	5	1.459 (30.42)	2.487 (315.63)	0.948 (8.31)	1.260 (19.36)	2.561 (373.72)	1.867 (88.26)
	15	1.337 (21.65)	2.385 (250.34)	0.682 (4.08)	1.226 (18.26)	2.458 (294.32)	1.725 (55.21)
	Mean	1.438 (28.23)	2.503 (331.25)	0.926 (8.57)	1.201 (17.08)	2.571 (385.13)	1.903 (89.51)
Goat (n = 18)	1	1.288 (19.26)	2.415 (280.75)	0.730 (5.27)	1.470 (30.89)	2.496 (336.18)	1.775 (62.78)
	2	1.341 (21.44)	2.492 (315.84)	1.037 (11.40)	1.235 (17.22)	2.558 (365.90)	1.959 (91.39)
	3	1.310 (19.90)	2.446 (279.44)	0.993 (9.61)	1.124 (12.69)	2.507 (321.63)	2.131 (139.76)
	4	1.227 (17.18)	2.427 (271.94)	0.939 (8.61)	1.074 (11.58)	2.482 (309.31)	2.081 (125.74)
	5	1.181 (14.57)	2.424 (266.38)	0.951 (8.82)	1.048 (10.56)	2.476 (300.32)	2.018 (105.95)
	15	0.970 (8.68)	2.161 (145.39)	0.852 (6.57)	0.802 (5.55)	2.219 (166.18)	1.513 (32.59)
	Mean	1.220 (16.84)	2.394 (259.96)	0.917 (8.38)	1.126 (14.75)	2.456 (299.92)	1.913 (93.04)
CV (%) ⁴		(28.9)	(22.0)	(48.2)	(44.8)	(20.6)	(28.4)
LSD _{0.05} ⁴		0.093	0.070	0.121	0.128	0.066	0.118

¹The values in parentheses correspond to the respective raw data means.²CMP: cytidyl-5'-monophosphate; UMP: uridyl-5'-monophosphate; GMP: guanosyl-5'-monophosphate; AMP: adenosyl-5'-monophosphate; total NT: total content of all nucleotides measured.³Mean: mean concentrations across lactation days.⁴The comparison of means was performed using the $\log_{10}(X + 1)$ transformed values.

Figure 1. A chromatogram from the analysis of a mixed standard nucleotide and nucleoside solution. Peaks: 1) cytidyl-5'-monophosphate (CMP); 2) uridyl-5'-monophosphate (UMP); 3) cytidine; 4) guanosyl-5'-monophosphate (GMP); 5) uridine; 6) adenosyl-5'-monophosphate (AMP); 7) inosine; 8) guanosine; and 9) adenosine. The arrow (10) indicates the elution time of orotic acid when absorbance readings are monitored at 278 nm.

sheep milk decreased up to the fourth day, exhibiting a maximum, though, on the third day, whereas its level on the fifteenth day was comparable to that on d 1 after parturition.

The significant reduction of NS concentration in goat milk ($54.33 \mu\text{mol/L}$) at 15 d (Table 2) was expected as

a result of the similar reduction patterns observed for uridine, cytidine, guanosine, and inosine (Figure 2). The fact that the concentration of pyrimidine NS (cytidine and uridine) in sheep milk was 1 order of magnitude higher than that of the purine NS (inosine, guanosine, and adenosine) at 4 d can be attributed to, among other things, the maximum concentration of cytidine recorded at that time, and mainly to the consistent increase of uridine with increased lactation day.

The evaluation of our findings on NS content in sheep and goat milk, in the context of the literature, is difficult because similar studies are rather limited. All individual NS were found in higher concentration in colostrum and transitional milk (averaged over 1–5 d) than in mature milk, with the exception of inosine and uridine in sheep milk. This is in agreement with the results of Schlimme et al. (1997, 2000), who found that the concentration of NS in the colostrum of most mammalian species was higher compared with that in the respective mature milk. The particular case of inosine is excluded from the above because it demonstrated no constant tendency across the lactation period. On the other hand, uridine was the only NS exhibiting much higher concentration levels on d 15 postpartum, concurring with Martin et al. (2005), who reported higher values for most NS in the mature milk. The overall increased uridine levels recorded in the present study agree with those found by Schlimme et al. (1997, 2000) in bovine milk (Figure 3). Concerning the lactobiochemical explanation for these

Figure 2. Effect of lactation day on uridine, total nucleoside (NS), cytidine, adenosine, guanosine, and inosine concentrations in sheep (▲) and goat (□) milks ($\mu\text{mol/L}$).

Figure 3. a) Nucleoside and b) nucleotide [guanosyl-5'-monophosphate (GMP), adenosyl-5'-monophosphate (AMP), cytidyl-5'-monophosphate (CMP), and uridyl-5'-monophosphate (UMP)] profile in sheep and goat milk compared with cow and human milk. Data for cow milk and human milk were adapted from ¹Schlimme et al. (1997), ²Duchén and Thorell (1999), ³Gil and Sanchez-Medina (1981), and ⁴Gill and Sanchez-Medina (1982).

findings, Schlimme et al. (2000) suggested as a possible reason the humoral origin, an enhanced local biosynthesis route, or both. Schlimme et al. (1997), examining the concentration levels and pattern of NS in bovine milk, reported the presence of uridine, cytidine, and low concentrations of adenosine, guanosine, and inosine, whereas in human milk they found uridine, cytidine, adenosine, and low concentration of guanosine. Instead, Duchén and Thorell (1999) found only cytidine and uridine in human milk, implying that the NS composition in human milk differs markedly from that in milk of ruminants (Figure 3).

Nucleotides

Regarding nucleotides, the ANOVA indicated that the milk species had a significant effect on AMP ($P = 0.024$), CMP, UMP, and total NT ($P < 0.001$), whereas it did not have any effect on GMP. In addition, the lactation day and the milk species $\times$ lactation day interaction had a significant effect on all parameters studied (Table 1). In particular, the total NT content (raw data averaged over lactation days) was $385.13 \mu\text{mol/L}$ in ovine milk compared with $299.92 \mu\text{mol/L}$ in caprine milk (Table 3). The concentrations of UMP and CMP, averaged over the sampling period, were also higher in ovine secretions (331.25 and $28.23 \mu\text{mol/L}$, respectively) compared with those in caprine secretions (259.96 and $16.84 \mu\text{mol/L}$, respectively). The same tendency was recorded for each sampling time as well. The NT AMP deviated from this pattern because its concentration was higher in caprine secretion at 1 d postpartum. The highest UMP concentration at 2 d after parturition for both milk species is in agreement with the results reported by Ferreira et al. (2001). The increased CMP and GMP concentrations at 2 d in caprine and at 4 and 3 d, respectively, in ovine milk are in contrast with the results reported by Schlimme et al. (1997), who instead found maximum concentrations for all NT at 1

d after parturition. The NT content in colostrum (1–3 d) was increased compared with mature milk (15 d) for both species. Similarly, Gil and Sanchez-Medina (1981) reported decreased NT levels with increasing lactation day, although the values recorded for ovine colostrum were much higher than those of the present study. It is also worth noting that the differences between lactation day means within each milk species were lower for most of the NS examined compared with the corresponding values obtained for the NT (Tables 2 and 3). The 3 and 4 times higher total concentration of NT compared with that of NS in caprine and ovine milk, respectively (Tables 2 and 3), combined with the higher total NT content in sheep milk than in goat milk, is in agreement with the results reported by Gil and Sanchez-Medina (1981). In contrast, Ferreira et al. (2001) observed lower NT levels in ovine milk (2 mo postpartum) compared with caprine milk. The fact that GMP was determined in all samples of our study does not comply with the findings of Gil and Sanchez-Medina (1981), who identified GMP only in goat milk, as well as with the results of Ferreira et al. (2001), who did not detect GMP in any of the milk samples examined. A wider range of NT concentrations (5.5 – $84.2 \mu\text{mol/L}$) was noted for human milk by Gil and Sanchez-Medina (1982), Janas and Picciano (1982), Thorell et al. (1996), and Duchén and Thorell (1999), whereas Ferreira (2003) found substantially higher NT concentrations ($162.9 \mu\text{mol/L}$) in human milk. The results of this study have shown an even higher NT content for ovine and caprine milk.

Complementary Remarks

The considerable NS or NT interindividual variation recorded in both milk species (Tables 2 and 3) and especially that of guanosine ($\text{CV} = 63\%$; Table 2) could be attributed to variability among individual animals. Similar substantial variation was reported by Schlimme et al. (1997), studying NS or NT content for bovine

milk, and by Leach et al. (1995) and Thorell et al. (1996) for human milk. The predominance of uridine and uridyl-5'-monophosphate in all samples, regardless of milk species and lactation day (Tables 2 and 3), is in agreement with the results reported by Schlimme et al. (1997) and Martin et al. (2005). In addition, the UMP levels obtained in the present study are similar with those found by Gil and Sanchez-Medina (1981) for colostrum of both species of milk.

The total purine (inosine + guanosine + adenosine + GMP + AMP) content (averaged over lactation days) was slightly higher in caprine milk (39.29 $\mu\text{mol/L}$) than in ovine milk (35.63 $\mu\text{mol/L}$). In contrast, a substantially higher total pyrimidine (cytidine + uridine + CMP + UMP) content (averaged over lactation days) was found in ovine milk (442.31 $\mu\text{mol/L}$) compared with caprine milk (352.89 $\mu\text{mol/L}$). The substantial predominance of pyrimidines in our study is in agreement with results reported for many ruminant milks and human milk by several researchers (Gil and Sanchez-Medina, 1981; Leach et al., 1995; Thorell et al., 1996; Boza, 1998; Martin et al., 2005). These data could be attributed to the higher catabolism of purines in the mammary gland compared with pyrimidines that seem to be better preserved (Tiemeyer et al., 1984; Thorell et al., 1996; Boza, 1998). However, Schlimme et al. (1997) reported equal levels for pyrimidine and purine NS in caprine milk.

For orotic acid, no significant effect of milk species was noted (Table 1). However, a careful examination of the interaction of milk species $\times$ lactation day indicated that its concentration decreased with advancing lactation day and reached the level of 55.21 and 32.59 $\mu\text{mol/L}$ for sheep milk and goat milk, respectively (Table 3). The orotic acid, a precursor of pyrimidine NT, was identified in all ovine and caprine samples analyzed, but in lower levels compared with bovine milk (Gil and Sanchez-Medina, 1981; Janas and Picciano, 1982; Tiemeyer et al., 1984; Ferreira, 2003). Orotic acid was not detected in human milk (Gil and Sanchez-Medina, 1982; Janas and Picciano, 1982; Thorell et al., 1996; Ferreira, 2003). Besides the absence of orotic acid, literature data indicate, as already mentioned, different contents of NS and NT in human milk (Gil and Sanchez-Medina, 1982; Janas and Picciano, 1982; Leach et al., 1995; Thorell et al., 1996; Schlimme et al., 1997; Duchén and Thorell, 1999) and cow milk (Gil and Sanchez-Medina, 1981; Tiemeyer et al., 1984; Schlimme et al., 1997; Figure 3).

Potential Applications

The interspecies differences in the concentration of cytidine and adenosine in the colostrum phase and uri-

dine in mature milk observed in this study may reflect the evolutionary adaptation of the neonates to different demands and could be of major biological importance for the clinical use of colostrum preparations or for the use of milk within a targeted pharmacological and metabolic framework. Recent findings obtained from nutritional studies indicate that the dietary nucleotides may affect the mucosal barrier, specifically through their effect on the sensing mechanism that involves purinergic signaling (Grimble and Westwood, 2001). For example, adenosine release by one organ as a response to stress can signal protective effects to other organs in a process called remote preconditioning (Grimble and Westwood, 2001). Other studies (Playford et al., 2000) further indicate that the bovine colostrum fractions might be useful for the treatment of a wide variety of gastrointestinal conditions, including inflammatory bowel disease, nonsteroidal antiinflammatory drug-induced gut injury, and chemotherapy-induced mucositis.

In view of the general consensus that colostrum preparations have the advantage of being easily accepted by patients as natural products, further studies are needed to elucidate the beneficial effect of caprine colostrum preparations in clinical management of patients with specific nutritional requirements. Likewise, sheep milk could be used as a natural alternative nutritional supplement in pre- and postoperative clinical nutrition because of its high uridine content and the synergistic effect of the NS and NT available.

CONCLUSIONS

The results of the present work allow the following conclusions to be drawn: 1) the total NT was higher than the total NS in both species of milk; 2) the colostrum of both species was substantially richer in NT than mature milk; 3) the uridine and uridyl-5'-monophosphate were the predominant constituents in the NS and NT pools of all samples, regardless of milk species and lactation day; 4) orotic acid was identified in all ovine and caprine samples analyzed; 5) uridine concentration was almost 3-fold higher in mature ovine milk compared with its caprine counterpart; and 6) the mean level of cytidine across the sampling period was much higher in sheep milk, whereas goat milk was on average far richer in adenosine.

REFERENCES

- Boza, J. 1998. Nucleotides in infant nutrition. *Monatsschr. Kinderheilkd.* 146:S39-S48.
- Carver, J. D. 1999. Dietary nucleotides: Effects on immune and gastrointestinal systems. *Acta Paediatr.* 430(Suppl.):83-88.
- Cosgrove, M. 1998. Nucleotides. *Nutr.* 14:748-751.
- Duchén, K., and L. Thorell. 1999. Nucleotide and polyamine levels in colostrums and mature milk in relation to maternal atopy and

- atopic development of the children. *Acta Paediatr.* 88:1338–1343.
- EC. 1996. European Commission Directive 96/4/EC amending Directive 91/321/EEC on infant formulae and follow-on formulae, 28/02/1996. *Off. J. L.* 049:12–16.
- Ferreira, I. M. P. L. V. O. 2003. Quantification of non-protein nitrogen components of infant formulae and follow-up milks: Comparison with cows' and human milk. *Br. J. Nutr.* 90:127–133.
- Ferreira, I. M. P. L. V. O., E. Mendes, A. M. P. Gomes, M. A. Faria, and M. A. Ferreira. 2001. The determination and distribution of nucleotides in dairy products using HPLC and diode array detection. *Food Chem.* 74:239–244.
- Gil, A., and F. Sanchez-Medina. 1981. Acid-soluble nucleotides of cow's, goat's and sheep's milks, at different stages of lactation. *J. Dairy Res.* 48:35–44.
- Gil, A., and F. Sanchez-Medina. 1982. Acid-soluble nucleotides of human milk at different stages of lactation. *J. Dairy Res.* 49:301–307.
- Gomez, K., and A. Gomez. 1984. *Statistical Procedures for Agricultural Research*. John Wiley and Sons Inc., Hoboken, NJ.
- Grimble, G. K., and O. M. Westwood. 2001. Nucleotides as immunomodulators in clinical nutrition. *Curr. Opin. Clin. Nutr. Metab. Care* 4:57–64.
- IDF. 2008. World Dairy Situation 2008. IDF Bulletin 432. International Dairy Federation, Brussels, Belgium.
- Janas, L. M., and M. F. Picciano. 1982. The nucleotide profile of human milk. *Pediatr. Res.* 16:659–662.
- Leach, J. L., J. H. Baxter, B. E. Molitor, M. B. Ramstack, and M. L. Masor. 1995. Total potentially available nucleosides of human milk by stage of lactation. *Am. J. Clin. Nutr.* 61:1224–1230.
- Martin, D., I. Clawin-Rädecker, P. C. Lorenzen, M. Ziebart, and K. Barth. 2005. Ribonucleosid-Gehalte in Schaf- und Ziegenmilch. *Kieler Milchwirtschaftliche Forschungsberichte* 57:21–32.
- Michaelidou, A., and J. Steijns. 2006. Nutritional and technological aspects of minor bioactive components in milk and whey: Growth factors, vitamins and nucleotides. *Int. Dairy J.* 16:1421–1426.
- Michaelidou, A. E. 2008. Factors influencing nutritional and health profile of milk and milk products. *Small Rumin. Res.* 79:42–50.
- Playford, R. J., C. E. MacDonald, and W. S. Johnson. 2000. Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. *Am. J. Clin. Nutr.* 72:5–14.
- Sánchez-Pozo, A., and A. Gil. 2002. Nucleotides as semiessential nutritional components. *Br. J. Nutr.* 87(Suppl. 1):S135–S137.
- Schlimme, E., D. Martin, and H. Meisel. 2000. Nucleosides and nucleotides: Natural bioactive substances in milk and colostrum. *Br. J. Nutr.* 84(Suppl. 1):S59–S68.
- Schlimme, E., D. Martin, H. Meisel, K. Schneehage, S. Hoffmann, E. Sievers, F. G. Ott, and K. P. Raetzke. 1997. Species-specific composition pattern of milk ribonucleosides and -nucleotides: Chemical and physiological aspects. *Kieler Milchwirtschaftliche Forschungsberichte* 49:305–326.
- Thorell, L., L. B. Sjöberg, and O. Hernell. 1996. Nucleotides in human milk: Sources and metabolism by the newborn infant. *Pediatr. Res.* 40:845–852.
- Tiemeyer, W., M. Stöher, and D. Giesecke. 1984. Metabolites of nucleic acids in bovine milk. *J. Dairy Sci.* 67:723–728.